FORM PTO-1390 (REV 10-96) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEYS DOCKET NUMBER: 1209-121P

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 08/NEW 981310

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE 96/00779	14 June 1996	16 June 1995

TITLE OF INVENTION: ULTRASENSITIVE IMMUNOASSAYS

APPLICANT(S) FOR DO/EO/US: LANDEGREN, U1f

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau). (WO 97/00446)
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    International Search Report (PCT/ISA/210)
    International Preliminary Examination Report (PCT/IPEA/409)
    One (1) sheet of formal drawing

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
NEW	PCT/SE 96/00779	1209-121P

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

Search Report has been prepared by the EPO or JPO **$930.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$1,070.00**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**

CALCULATIONS PTO USE ONLY	

ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,070.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	11 - 20 =	0	X **$22.00**	$	
Independent claims	2 - 3 =	0	X **$82.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	Yes		+ **$270.00**	$	270.00
TOTAL OF ABOVE CALCULATIONS =				$	1,340.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	1,340.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	1,340.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$	1,340.00
				Amount to be: refunded	$
				charged	$

a. [X] A check in the amount of $ 1,340.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
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MURPHY, GERALD M., JR.
NAME

28,977
REGISTRATION NUMBER

/gfo 12/16/1997

PATENT
1209-0121P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT: LANDEGREN, Ulf

INT'L. APPLN. NO.: PCT/SE 96/00779

SERIAL NO.: GROUP:

FILED: December 16, 1997 EXAMINER:

FOR: ULTRASENSITIVE IMMUNOASSAYS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box Patent Applications
Washington, D.C. 20231

December 16, 1997

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

IN THE SPECIFICATION:

Change Title from "IMMUNOASSAY AND KIT WITH TWO REAGENTS THAT ARE CROSS-LINKED IF THEY ADHERE TO AN ANALYTE" to --ULTRASENSITIVE IMMUNOASSAYS--.

Before line 1, insert --This application is the national phase under 35 U.S.C. §371 of prior PCT International Application No. PCT/SE 96/00779 which has an International filing date of June 14, 1996 which designated the United States of America, the entire contents of which are hereby incorporated by reference.--

IN THE CLAIM:

CLAIM 5: Lines 1 and 2, change "any of the claims 1-4" to --claim 1--.

R E M A R K S

The specification has been amended to provide a cross-reference to the previously filed International Application.

The above amendment to the claim corrects the improper multiple dependency, and places the application into better form prior to examination.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

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GMM/gfo

IMMUNOASSAY AND KIT WITH TWO REAGENTS THAT ARE CROSS-LINKED IF THEY ADHERE TO AN ANALYTE

Technical field

The present invention relates to ultrasensitive immunoassays. More specifically, it relates to immunological test kits and processes for immunological detection of a specific antigen. In the present invention, the fields of immunology and molecular genetics are combined.

Background of the invention

Immunoassays represent powerful tools to identify a very wide range of compounds, such as antigens and antibodies. Examples of immunoassays are ELISA (enzyme linked immunosorbent assay), EIA (enzyme immunoassay), and RIA (radio immunoassay). Common to all these immunoassays, is that detection sensitivity is limited by the affinity of typical antibodies.

With the prior art immunoassays, detection is not possible below a certain number of molecules, because the background, i.e. unspecifically bound material, interferes with the results. Detection of very low numbers of antigen is becoming increasingly important, especially for diagnostic applications. Therefore, further developments in sensitivity as well as specificity of immunological assays are desired.

Cantor et al, Science, Vol. 258, 2 Oct. 1992, have previously reported the attachment of oligonucleotides to antibodies in order to permit detection of such antibodies having bound antigen in immune reactions. A streptavidin-protein A chimera that posseses tight and specific binding affinity for both biotin and immunoglobulin G was used to attach biotinylated DNA specifically to antigen-monoclonal antibody complexes that had been immobilized on microtiter plate wells. Then, a segment of the attached DNA was amplified by PCR (Polymerase Chain Reaction). Analysis of the PCR products by agarose gel electrophoresis after staining with ethidium bromide allowed detection of 580 antigen molecules ($9,6 \times 10^{-22}$ moles) which is a significant improvement compared

to, for example, conventional ELISA.

However, in Cantor et al., the labeled DNA-antibody complexes are assembled in situ during the assay. This can create variable stoichiometry in the assembly of the components and in the attachment of the DNA label. Moreover, extra steps are required for addition of biotinylated reagents and binding proteins. Numerous wash steps are also needed to remove excess reagents and to free assay components of non-specifically bound reagents.

Hendrickson et al., Nucleic Acids Research, 1995, Vol 23, No.3, report an advancement of the Cantor et al. assay that reduces complexity. This is achieved through labeling antibody with DNA by direct covalent linkage of the DNA to the antibody. In this approach, the analyte specific antibody and the 5' amino modified DNA oligonucleotide are independently activated by means of separate heterobifunctional cross-linking agents. The activated antibody and DNA label are then coupled in a single spontaneous reaction.

International patent publication no. WO 91/17442 describes a molecular probe for use as a signal amplifier in immunoassays for detecting i.a. antigens. The probe comprises an antibody, a double stranded polynucleotide functioning as a promoter for a DNA dependend RNA polymerase, and a single or double stranded template for the promoter. The transcription product is quantified and correlated to the amount of present antigen in a sample.

However, in all three of the above described immunoassays the attached DNA is only used as a marker by being amplified to detectable levels. There is no distinction between oligonucleotides attached to antibodies having bound antigen and oligonucleotides attached to antibodies not having bound antigen, i.e. those being non-specifically trapped. Non-specifically trapped antibodies give rise to an undesired background signal and limits the minimun number of antigen molecules that can be detected and it will not be possible to distinguish between false positive and

true positive results below a certain number of antigen molecules. Commonly, solid supports such as microtiter plates, are used for the reactions. According to prior art, there will always be an excess of oligonucleotide-labeled antibody that cannot be removed from the solid support by adding background-lowering agents and by repeated wash steps.

Summary of the invention

The present invention enables detection of extremely low numbers of antigenic molecules, even down to a single molecule. The invention provides reliable immunoassays in situations where insufficient numbers of antigens are available for conventional assays.

According to a first aspect of the invention, there is provided an immunological test kit comprising a first immobilized reagent having affinity for a specific macromolecule, such as a protein. Furthermore, the test kit comprises a second and a third affinity reagent specific for different determinantes of said macromolecule, and modified with crosslinkable compounds enabling a) conjugation of said second and third affinity reagent only when both are bound to the said, same macromolecule, and b) detection by amplification.

According to a preferred embodiment of the invention, the affinity reagents are antibodies and the crosslinkable compounds are oligonucleotide extensions attached to the second and third antibody, respectively. The macromolecule is in this case a specific antigen.

According to a second aspect of the invention there is provided an immunoassay for detection of a specific antigen, comprising the following steps:

a) contacting a sample suspected of containing said specific antigen with a first antibody linked to a solid support, said first antibody being specific for a first epitope on the antigen,

b) washing off excess reagents,

c) incubating with a solution of a second and a third antibody specific for a second and third epitope of said antigen, and modified with crosslinkable oligonucleotides enabling conjugation of said second and third antibody when both are bound to the said, same antigen,
d) washing off excess reagents,
e) amplifying said crosslinked oligonucleotides, and
f) detecting the amplified products.

Products from the amplification reaction only result when two antibodies, i.e. the second and the third, have bound to the same antigen. Thus, amplification is specific for antibodies having bound to antigen. Non-specifically trapped antibodies do not give rise to any signal.

Detailed description of the invention

The present invention will be described more detailed below with reference to the accompanying drawings, in which

Fig. 1 is a schematic view of the principles of the immunoassay according to the invention, and

Fig. 2 shows chemical coupling of amino-modified oligonucleotides to macromolecules.

In Fig. 1 there is shown an immobilized antibody to a specific antigen applied together with two other antibodies, specific for other determinants on the same antigen. Besides antibodies other specifically interacting species with a known affinity, such as lectins, receptors, single chain antibodies, cofactors, oligonucleotides and other non-proteins, can be used in the invention.

The interacting species are modified with crosslinkable compounds in the form of an interacting pair, preferably short oligonucleotide extentions. Upon the coordinated binding of several so modified antibodies, oligonucleotides of neighbouring antibodies are conjugated to each other. The conjugation may or

may not necessitate an enzymatic ligation step depending on the orientation of the oligonucleotide extensions.

If the conjugation is between free 3' and 5' ends ligation is necessary, such as by T4 RNA ligase or T4 DNA ligase. To facilitate the conjugation, it is convenient to use a stretch of oligonucleotides base pairing to and, thereby, juxtaposing the free ends of the oligonucleotides and permitting their joining through ligation.

If the conjugation is between free 3' ends these have to be designed to be mutually complementary to achieve base pairing and initiation of DNA synthesis extending the 3' ends of the the molecules.

Thus, only in those cases where the antibodies are brought close enough through binding to the same antigen molecule can the oligonucleotides be ligated. Ligated molecules subsequently serve as templates for nucleic acid amplification reactions.

In Fig. 2, there is shown a suitable way to attach the oligonucleotide extension to the antibodies. First, the oligonucleotides are terminally amino-modified and then attached to primary amines on the antibodies via disulphide bonds, e.g. according to the technique of Chue and Orgel, Nucleic Acid Research, Vol. 16, No. 9, 1988. Another way is by direct covalent coupling as described by Hendrickson et el., supra.

The antibodies used in the invention can be polyclonal, monoclonal or single chain antibodies produced by bacteriophages. In the latter case, it is possible to have antibodies equipped with an oligonucleotide binding part, rendering the above coupling step between antibody and oligonucleotide unnecessary.

The amplification technique to obtain detectable products is, for example, PCR (Polymerase Chain Reaction), LCR (Ligase Chain Reaction), SDA (Strand Displacement Amplification) bacteriophage

Qβ replication, and 3SR (Self-Sustained Synthetic Reaction), of which the latter three methods do not require temperature cycling.

The method for detecting amplified products can, for example, be direct incorporation of a label, such as radioisotopes, fluorochromes, and enzymes, into the amplified products with the use of label-conjugated primers or nucleotides. Preferably, the accumulation of amplified products is monitored via the fluorescence from intercalating dyes, such as propidium iodide, etidium bromide and SYBR™ green from Molecular Probes.

The invention is not restricted to detection of any special kind of macromolecule, such as an antigen; the only criterion it has to fulfil is that it must be able to simultaneously bind three antibodies/affinity reagents. In the case where the affinity reagents are antibodies, the three antibodies are specific for different epitopes on the antigen. By biosensor analysis, it is possible to assure that the antibodies do not bind to overlapping epitopes on the antigen.

Examples of macromolecules are human myoglobin and human growth hormone. Ultrasensitive assays for growth hormone will have significant value in clinical situations where hormone levels are undetectable by prior art assays.

The invention will now be described below in a non-limiting Example.

EXAMPLE

Immunoglobulins were modified in a reaction with SPDP (3-(-pyridyldithio)propionic acid N-hydroxysuccinimide ester, from Pharmacia Biotech) according to the manufacturer's suggestions. Oligonucleotides were thiolated, either through the addition of a suitable phosphoramidite according to Connolly (Connolly BA, Nucl. Acid. Res. 1987 15:3131), or 3'aminomodified oligonucleo-

tides were reacted with SPDP, followed by reduction of the dithiopyridyl bond, using dithiothreitol.

SPDP-modified antibodies were incubated with three equivalents of SH-containing oligonucleotides at 4°C over night. The reaction mixture was separated using a Zorbax HPLC gel filtration column. Residual free antibody were removed from the isolated conjugate by ion exchange MonoQ FPLC separation.

The two oligonucleotides used to conjugate the antibodies were Oligo 1: 5'Tr S C3-ATA GAC TGA GCG TGG ACA TTA ATA TGT ACG TAC GCT TAA TTG AGT 3' and Oligo 2: 5'P ATG TAC GAC CCG TAG ATA TTA TCA TAC TGG CAT GGG CAT GAT GAA CAT C-NHSPDP T3'

The immune test was performed by first binding 1µg of biotinylated antibody (#1) to individual streptavidin-coated prongs on a manifold support. [Parik et al., Anal. Biochem; (1993) 211: 144-150B]. After washes using PBS (phosfhate buffered saline) with 0.5% Tween 20, the prongs were lowered into solutions of antigen (myoglobin) at variable concentrations. After further washes, the supports with bound antigen were incubated in a solution of two oligonucleotide-conjugated antibodies #2 and #3 at 5 ng each per reaction. The supports were washed, an oligonucleotide complementary to the free ends of the antibody-conjugated oligonucleotides was added (4 pmol per reaction, 5'CTA CGG GTC GTA CAT ACT CAA TTA AGC GTA 3'), and the ends of oligonucleotides on nearby antibodies were joined covalently by ligation at 37°C for 30 min using 1 U of T4 DNA ligase. The supports were then washed in a standard PCR buffer, and the supports were added as templates in a PCR mix, including two primers specific for sequences located at either side of the ligation junction (5'TTA ATG GCG AG 3') and Taq polymerase. After two cycles, the supports were removed and the amplification was continued for 26 more cycles. Amplification products were examined by separation in an agarose gel and ethidium bromide staining.

CLAIMS

1. An immunological test kit comprising a first immobilized reagent having affinity to a specific macromolecule, characterized in a second and a third affinity reagent specific for different determinantes of said macromolecule, and modified with crosslinkable oligonucleotides.

2. An immunological test kit according to claim 1, characterized in that the affinity reagents are antibodies, and that the macromolecule is a specific antigen.

3. An immunological test kit according to claim 1, characterized in that the affinity reagents are lectins, receptors, single chain antibodies, cofactors and nucleic acids.

4. An immunological test kit according to any one of the claims 1-3, characterized in that the oligonucleotides are complementary to each other.

5. An immunological test kit according to any of the claims 1-4, characterized in that it further comprises a ligase.

6. An immunoassay for detection of a specific antigen, characterized in:

a) contacting a sample suspected of containing said specific antigen with a first antibody linked to a solid support, said first antibody being specific for a first epitope on the antigen,

b) washing off excess reagents,

c) incubating with a solution of a second and a third antibody specific for a second and third epitope of said antigen, and modified with crosslinkable oligonucleotides enabling conjugation of said second and third antibody when both are bound to the said, same antigen,

d) washing off excess reagents,

e) amplifying said crosslinked oligonucleotides, and

f) detecting the amplified products.

7. An immunoassay according to claim 6, characterized in that a ligase is added before step d).

8. An immunoassay according to claim 6 or 7, characterized in that an oligonucleotide complementary to the crosslinkable oligonucleotides is added before step d).

FIG. 1

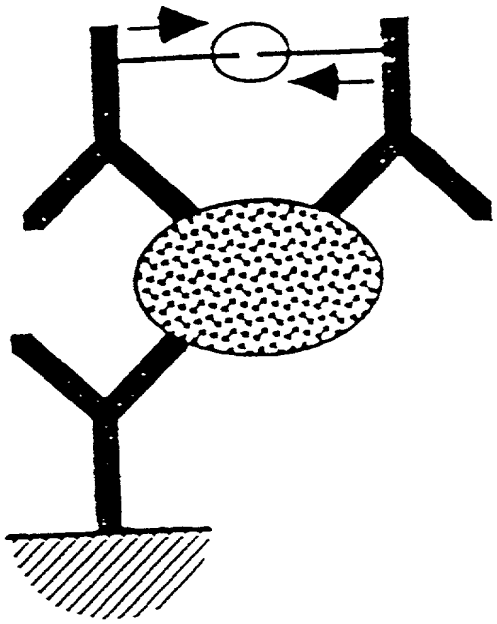
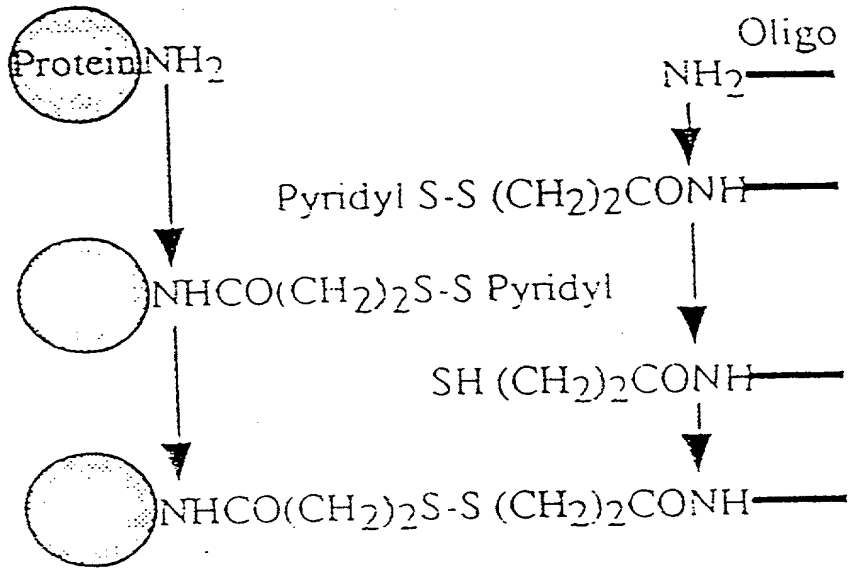


FIG. 2



PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO. 1209-121P

As a below named inventor, I hereby declare that: my residence post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: * Ultrasensitive immunoassays

Insert Title

Check Box If Appropriate — For Use Without Specification Attached

the specification of which is attached hereto unless one of the following boxes is checked:

☐ The Specification was filed on _____ and was assigned Serial No. _____ and was amended on _____

☒ was filed as PCT international application number PCT/SE96/00779 on June 14, 1996 and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows:

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below:

Insert Priority Information (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
9502196-0 (Number)	Sweden (Country)	06.16.1995 (Month/Day/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Country	Application No.	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status — patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status — patented, pending, abandoned)

*NOTE: Must be completed.

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor: Insert Name of Inventor Insert Date This Document Is Signed Insert Residence Insert Citizenship Insert Post Office Address

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	*DATE
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Full Name of Third Inventor, if any: see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	*DATE

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Full Name of Fourth Inventor, if any: see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	*DATE

RESIDENCE (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Fifth Inventor, if any: see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	*DATE

RESIDENCE (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

*Note: Must be completed — date this document is signed.

(USPTO Approved 3-90)
(Revised 3-92)